



zoon. This in turn is lost about $60 \pm 0.5 \mu\text{m}$ behind the head and the flagellum assumes a smooth tubular form for the remainder of the spermatozoan length.

Lymnea peregra (Müller, 1774)

The mature spermatozoon of *Lymnea peregra* is $690 \pm 5 \mu\text{m}$ long. The spermatozoan head is similar to that of *Arion* in that it describes only one gyre but differs in that it consists of 7 parallel helices (Figure 8). The head is $5.5 \pm 0.2 \mu\text{m}$ long and is surmounted by a digitiform acrosome (Figure 8). The 7 parallel helices are continued into the mitochondrial derivative (Figure 8). These helices have a pitch of $4.5 \pm 0.2 \mu\text{m}$ and run for a distance of $30 \pm 1 \mu\text{m}$ behind the head. Just behind the head it is difficult to detect any disparity in size of the helices but slightly posterior it can be seen that 2 of the helices are larger than the other 5 (Figure 9, arrows). These 2 larger helices may be termed major helices since thin sections show that they contain glycogen deposits.

The 5 smaller or minor helices are lost $30 \mu\text{m}$ behind the head and only the 2 major helices continue to run posteriorly, at this stage with a pitch of $3.0 \pm 0.1 \mu\text{m}$ and for a distance of $400 \pm 5 \mu\text{m}$ along the flagellum (Figure 10). The helical organisation is lost completely towards the tail tip with the result that a tubular form is assumed for the distal $260 \pm 5 \mu\text{m}$ of the gamete length. This portion of the flagellum is considerably smaller in diameter than the more anterior regions (Figure 10, arrow).

Helix aspersa Müller, 1774

The total length of the mature spermatozoon is $660 \pm 5 \mu\text{m}$. The gross form of this spermatozoon is simpler than the others described and differs quite markedly from them. The head is $5.5 \pm 0.2 \mu\text{m}$ long appearing smooth and tapering anteriorly (Figure 11) although SEM preparations indicate that the head surface may be slightly wrinkled but this may be due to contamination of the specimen. There is a single large helix in the mitochondrial derivative which has a pitch of $23.5 \pm 0.5 \mu\text{m}$ and is flattened around the core of the flagellum so that it is difficult to detect in the SEM (Figure 11). The helix runs over

$400 \mu\text{m}$ of the spermatozoan length but is gradually flattened around the flagellum throughout this distance with the result that the tail usually appears smooth and tubular in form.

DISCUSSION

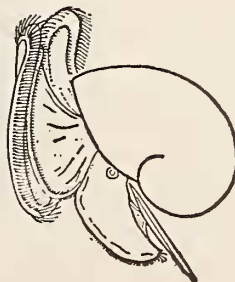
The application of freeze-drying and study of whole cells in the SEM allows a rapid appreciation of the form of the complex spermatozoa of the pulmonate molluscs with particular emphasis on the precise layout of the mitochondrial helices and any variation in their form throughout the gamete length. It is apparent from the small number of species here examined that there is considerable variation, not only in overall length of spermatozoon, but also in the number and disposition of helical structures present and that pulmonate spermatozoa are not in fact practically identical. It is important that further work be carried out in this field, also at a more detailed level, to catalogue the variation in spermatozoan form between species, genera and families within the pulmonates.

ACKNOWLEDGMENTS

I am grateful to Mr A. B. Britton of the Department of Biochemistry and Mr C. Hassett of the Botany Department, University of Bristol, for technical assistance during this study.

Literature Cited

- BACCETTI, BACCIO & A. G. BURRINI
1973. An improved method for the scanning electron microscopy of spermatozoa. *Journ. Microscopy* 99 (1): 101-109 (Sept. 1973)
- BAYNE, CHRISTOPHER JEFFREY
1970. Organisation of the spermatozoon of *Agriolimax reticulatus*, the Grey Field Slug (Pulmonata, Stylommatophora). *Zeitschr. Zellforsch.* 103: 75-89
- THOMPSON, THOMAS EVERETT
1971. Application to molluscan ultrastructure research of the Balzers 360 M freeze etching plant. *The Veliger* 13 (4): 367-368; 1 pl. (1 April 1971)
1973. Euthyneuran and other molluscan spermatozoa. *Malacologia* 14: 167-206



Fine Structure of the Sperm of the Freshwater Clam

Ligumia subrostrata (Say, 1831)¹

(Mollusca : Bivalvia)

BY

JOHN J. TRIMBLE III AND DAVID GAUDIN

Department of Zoology and Physiology, Louisiana State University
Baton Rouge, Louisiana 70803

(3 Plates)

INTRODUCTION

FINE STRUCTURAL STUDIES of pelecypoda sperm are sparse, and the majority of these studies have been done on sperm of the marine forms. To the authors' knowledge no reports exist on the fine structure of sperm from a freshwater bivalve. The clam, *Ligumia subrostrata* (Say, 1831), is a common inhabitant of freshwater ponds and marshes of the gulf coast area. The fine structure of the sperm of this species was studied to contribute additional information about the nature of primitive spermatozoan morphology.

MATERIALS AND METHODS

Clams used for this study were collected from a small freshwater pond in southern Louisiana. Mature sperm and sperm packets were removed from the clam by transection of the gonad. Tissue was quickly immersed in cold 3% glutaraldehyde, buffered with 0.1 M Millonig's phosphate buffer (pH 7.3), to which 1 mM CaCl₂ and 2% sucrose

were added. After a 3 hr fix, tissue was washed overnight in buffer, post fixed for 1 hr in cold 1% osmium tetroxide in buffer, rinsed with distilled water, dehydrated in alcohol and embedded in Spurr's epoxy. Sections were cut on a Sorvall MT-2 ultramicrotome using glass knives, stained with alcoholic uranyl acetate and lead hydroxide, and viewed with either a Hitachi 11A or RCA-EMU-3G electron microscope.

Carbon replicas were prepared using glutaraldehyde fixed sperm. A drop of sperm suspension was applied to a parlodion coated grid and a few seconds were allowed for the sperm to settle out of solution onto the grid. Excess fluid was removed by blotting on filter paper. Grids were coated with about 100 Å thickness of carbon and the tissue digested with a solution of potassium permanganate and potassium dichromate in sulfuric acid (DAWES, 1971). Grids were washed in distilled water, dried and shadowed using a carbon-platinum pellet.

OBSERVATIONS

Sperm from *Ligumia subrostrata* measure 35 μm long and possess the classical head, midpiece and tail arrangement

¹ This work was supported in part from a grant from the Louisiana University Council on Research

Explanation of Figures 1 to 3

Figure 1: Carbon replica of mature sperm. Arrow indicates midpiece containing mitochondria × 24 000

Figure 2: Anterior tip of head of sperm showing acrosomal region (arrow) × 68 000

Figure 3: Longitudinal section through sperm showing nucleus (N), mitochondria (M) of midpiece, projecting into subnuclear fossa (white arrow), ring centriole (R) and axoneme (AX)

× 45 000